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Effect of Sulfur Nutrition on the Redistribution of Sulfur in Vegetative Soybean Plants[1]

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Soybean (*Glycine max* L.) plants were grown with sulfate at 2 (S_2) or 20 μM (S_{20}) and treated with [^{35}S]sulfate between d 36 and 38. Growth was continued with or without 20 μM sulfate (i.e. $S_2 \rightarrow S_0$, $S_2 \rightarrow S_{20}$, etc.). When the leaves of $S_{20} \rightarrow S_{20}$ plants were 70% expanded, they exported S and ^{35}S label from the soluble fraction, largely as sulfate, to new expanding leaves. However, ^{35}S label in the insoluble fraction was not remobilized. Very little of the ^{35}S label in the soluble fraction of the leaves of $S_{20} \rightarrow S_0$ plants was redistributed; most was incorporated into the insoluble fraction. The low levels of S remobilization from the insoluble fraction were attributed to the high level of N in the nutrient solution (15 mM). Most of the ^{35}S label in S_2 plants at d 38 occurred in the soluble fraction of the roots. In $S_2 \rightarrow S_0$ plants the ^{35}S label was incorporated into the insoluble fraction of the roots, but in $S_2 \rightarrow S_{20}$ plants ^{35}S label was rapidly exported to leaves 3 to 6. It was concluded that the soluble fraction of roots contains a small metabolically active pool of S and another larger pool that is in slow equilibrium with the small pool.

S is generally regarded as an immobile element in plants, since S-deficiency symptoms appear first in young leaves (Mengel and Kirkby, 1982; Salisbury and Ross, 1992). However, it is now evident from pulse-chase radiochemical labeling studies that endogenous S is redistributed from mature leaves of S-sufficient plants of subterranean clover (Bouma, 1966), tobacco (Rennenberg et al., 1979), siratro (Clarkson et al., 1983), soybean (Smith and Lang, 1988), wheat (Cooper and Clarkson, 1989), barley (Adiputra and Anderson, 1992), and tobacco (Herschbach and Rennenberg, 1994). In barley, some of the S that is initially distributed to expanding leaves is redistributed when the leaves approach and attain full expansion to other plant parts. Clarkson and colleagues (Clarkson et al., 1983; Cooper and Clarkson, 1989; Larsson et al., 1991) have proposed that redistribution of S from fully expanded leaves of siratro and cereals involves selective loading of soluble S compounds into the phloem and basipetal transport to the root system, followed by reloading into the xylem for recirculation in the transpiration stream to other leaves in the shoot system.

The redistribution of many elements from leaves of whole plants can be induced or enhanced by discontinuing the supply of the element (i.e. nutritional stress). For example, when wheat plants are deprived of exogenous N, the redistribution of endogenous N from old leaves into developing grains is enhanced (Simpson et al., 1983). Similar results have been described for leaf-to-leaf redistribution of P and K (Cram, 1990) and N (Pate, 1980). However, redistribution of S from mature leaves is not enhanced by S stress, as shown in studies with subterranean clover (Bouma, 1966), siratro (Bell et al., 1990), and barley (Adiputra and Anderson, 1993, 1995). More recently, Bell et al. (1995) reported that the insoluble S in mature leaves of siratro decreased by about 50% after 7 d when the sulfate supply was discontinued. This suggests that insoluble S is mobilized from mature leaves into other plant parts. However, it is not clear whether this is a temporal response or a response to S stress, since measurements of plants that were maintained at a constant level of S were not reported.

In this paper we report the effect of S nutrition on S redistribution in vegetative soybean (*Glycine max* L.) using the pulse/chase radiochemical labeling technique used previously (Sunarpi and Anderson, 1996). We also report the measurements of total, soluble, and insoluble S using ICP-OES and of sulfate in the constituent plant parts. The data indicate that, in the presence of 15 mM N and adequate S, insoluble S is not remobilized extensively, although this is enhanced to a small extent by S stress. However, soluble S is extensively redistributed from mature leaves to new developing leaves.

MATERIALS AND METHODS

Growing Conditions

The experimental design is summarized in Figure 1. Soybean seeds (*Glycine max* L. var Stephens) were germinated during the winter in a greenhouse in sand:vermiculite (2:1, v/v) and watered daily with deionized water. In the ensuing protocol, all times refer to days after imbibition (d 0). On d 17, the seedlings were transplanted into light-proof 1-L pots (two seedlings per pot) containing full-strength nutrient solution as described by Adiputra and Anderson (1992), except that 0.05 mM Fe-EDTA was used instead of Fe-citrate and S was supplied as $MgSO_4$ either at 2 or 20 μM (Fig. 1). These levels, which are referred to as S_2 and S_{20}, respectively, were intended to give a deficient (S_2) and an optimum (S_{20}) S supply. All trace elements were supplied as their chlorides and Mg^{2+} was maintained at 3

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Abbreviations: H1, harvest 1; H2, harvest 2, etc.; ICP-OES, inductively coupled plasma–optical emission spectrometry; L1, leaf 1; L2, leaf 2; etc.

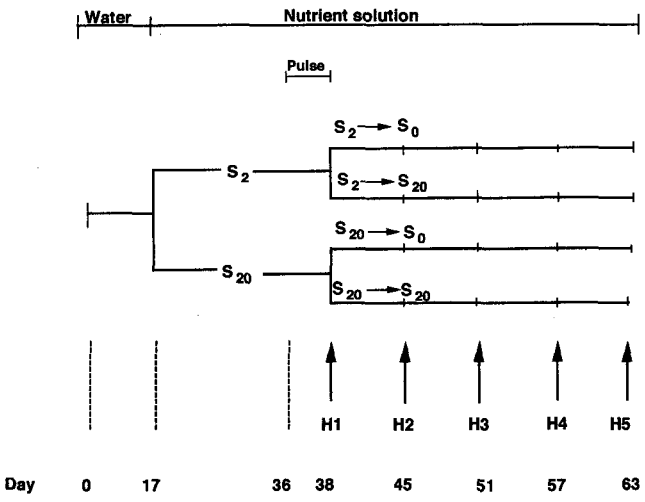

Figure 1. Summary of the experimental design showing the S nutrition treatments before, during, and after the pulse period. H1 to H5 and other aspects of the design are also shown. All times refer to the period (in days) after imbibition.

mM by addition of $MgCl_2$. The plants were grown under these conditions for a further period of 19 d (i.e. until d 36).

The length of the central leaflet of the trifoliate leaves was measured daily on a batch of sample plants (Fig. 2). All lateral branches were excised as they emerged but all leaves on the main stem were left intact. At d 36, each pot was supplied with 370 kBq (10 μCi) of $Na_2{}^{35}SO_4$ per L of nutrient solution containing 2 and 20 μM sulfate (i.e. specific radioactivity 185 and 18.5 GBq mol^{-1}, respectively). After 48 h (d 38), each pot was drained and the root system rinsed four times with deionized water. Half of the plants that had previously been grown at S_2 and S_{20} were then transferred into pots containing nutrient solution without sulfate (i.e. $S_2 \rightarrow S_0$, $S_{20} \rightarrow S_0$); the remainder were transferred to pots containing nutrient solution with 20 μM sulfate ($S_2 \rightarrow S_{20}$, $S_{20} \rightarrow S_{20}$). These solutions were aerated and replaced every 6 d for the remainder of the experiment. At d 38, eight plants (from four pots) were harvested (H1) and each plant was dissected into separate leaves (L1-L5), stems, and roots; at no stage were two individual plants from one pot combined. The plant materials were freeze-dried at −40°C for 3 d. The dried samples for each of the eight replicates were weighed individually, ground into fine powders, and stored in glass vials at room temperature until required. Further harvests (eight plants) were made on d 45 (H2), d 51 (H3), d 57 (H4), and d 63 (H5). The harvest dates, at 6-d intervals, coincided with the times when the nutrient solutions were replaced.

Determination of Total, Soluble, and Insoluble S and of Sulfate in Plant Materials

To obtain total S, the freeze-dried powders of each plant part from two plants from separate pots (i.e. two replicates) were digested and analyzed using ICP-OES as described previously (Sunarpi and Anderson, 1995). Soluble S was extracted from plant material (100 mg of powder) with 2 mL of 80% (v/v) ethanol at 70°C for 20 min. After centrifugation (5000*g*, 10 min), 0.5 mL of the supernatant solution (referred to as the ethanolic extract) was evaporated to dryness at 100°C for 15 min. The dried material was redissolved in 1 mL of 6 M HCl and incubated at 80°C for 10 min, and the volume was adjusted to 5 mL. Soluble S was then determined by ICP-OES as above. Insoluble S was estimated by subtracting soluble S from total S. Sulfate was determined by analyzing samples (50 μL) of the ethanolic extract by conductivity after passage through an HPLC fitted with an ion-exchange column (PRP-X100, Reno, NV) as described by Anonim (1995). The total, soluble, and insoluble S and sulfate content of each sample was adjusted by the dry weight of the plant part and is expressed as μmol (plant part)$^{-1}$.

Determination of ^{35}S Label in Plant Materials

Total ^{35}S label of samples (10 mg) of the powders of the plant parts from one of the two plants in each of the four pots (i.e. four replicates) was determined as described by Adiputra and Anderson (1992). The ^{35}S label associated with the soluble fraction was determined by pipetting 100 μL of the ethanolic extract, prepared as described above, into scintillation vials and counting. The ^{35}S label associated with the insoluble fraction was calculated by determining the difference between the total ^{35}S label and the ^{35}S label in the soluble fraction. The ^{35}S label present in each sample was corrected for radiochemical decay from a common date. All values were adjusted for dry weight and are expressed as kBq (plant part)$^{-1}$. Specific radioactivity was determined from the ratio of the ^{35}S label and the S content and is expressed as GBq mol^{-1}.

RESULTS

The plants that were grown initially at S_2 were just beginning to show evidence of S-deficiency symptoms (lamina and yellowed petiole) in L4 at the time the pulse was applied at d 36. Nonetheless, at the mid-time of the pulse, the leaf length of plants grown at 2 mM sulfate was only slightly less in expanding L4 and L5, compared with plants grown at S_{20} (Table I). The final leaf length and dry weight of L1 to L3 at the conclusion of the experiment were not influenced by the level of S nutrition during the period

Table I. *Leaf length and leaf expansion of L1 to L7 at H1, immediately after terminating the pulse, for plants grown in nutrient solution containing S_2 and S_{20}*

Plant Part	Leaf Length (mean ± SE)		Leaf Expansion	
	S_2	S_{20}	S_2	S_{20}
	cm		*%max*	
L1	4.9 ± 0.2	5.0 ± 0.2	100	100
L2	8.7 ± 0.3	8.7 ± 0.5	100	100
L3	8.6 ± 0.4	8.9 ± 0.4	100	100
L4	6.6 ± 0.3	7.7 ± 0.3	71	74
L5	1.4 ± 0.1	2.2 ± 0.1	17	21
L6	ndv[a]	ndv	ndv	ndv

[a] ndv, Not developed.

after the pulse. However, the final leaf length of the remaining leaves was strongly influenced by the level of S nutrition. For $S_2 \rightarrow S_{20}$ plants, the final leaf lengths of L4 to L6 were about 12.5, 24.4, and 69.2% longer, respectively, than those of $S_2 \rightarrow S_0$ plants (Fig. 2). The dry weight of the leaves exhibited a similar response (data not shown). Conversely, the final leaf lengths of L4 to L6 of $S_{20} \rightarrow S_0$ plants were 6, 22, and 44% less, respectively, than those for the corresponding leaves of $S_{20} \rightarrow S_{20}$ plants (Fig. 2); the corresponding decreases for final dry weight were 5, 33, and 50%, respectively.

Effect of S Nutrition on the Soluble and Insoluble S Pools and the Distribution of ^{35}S Label in the Plant Parts at H1

About 67% of the S in the S_2 plants occurred in the leaves at H1 and was more or less uniformly distributed between L1 and L4 (Fig. 3). In L3 (and presumably in the other mature leaves, L1 and L2) nearly all of the S occurred in the insoluble fraction (Table II), but in the expanding L4 about 37% of the S occurred as soluble S, mostly as sulfate (Table III). L5, which was in the very early stages of leaf expansion at H1 (Table I), contained relatively little S but about 46% occurred in the soluble fraction, mostly as sulfate. The root system accounted for about 22% of the total plant S, of which 17% occurred in the soluble fraction. Conversely, nearly all of the ^{35}S label in the S_2 plants at H1 occurred in the roots (Table IV), of which about 68% occurred in the soluble fraction (Table II). For S_2 plants the total amount of S per plant at H1 (41.1 μmol) was far in excess of the sulfate-S supplied in the nutrient solution up to this time (4 μmol per plant). Presumably, the balance was derived from the cotyledons, which provide a source of S for seedling growth, amounting to about 40 μmol per seedling (Sunarpi and Anderson, 1995).

At H1 the S_{20} plants differed significantly from the S_2 plants in several ways. Although the S contents of L1 and L2 were similar to those of S_2 plants, the S contents of all of the other plant parts, especially L4, were much higher in the S_{20} plants (Fig. 4). All of the leaves of the S_{20} plants had a higher content of insoluble S than the S_2 plants (Tables II and V). However, with the possible exception of L5, which was in an early stage of development, the proportion of S in the soluble fraction of each plant was much higher in the S_{20} plants. This included mature structures such as L3, as well as rapidly developing plant parts, such as L4. In S_{20} plants L4, which was 70% expanded at H1, contained a very high level of soluble S, mostly as sulfate.

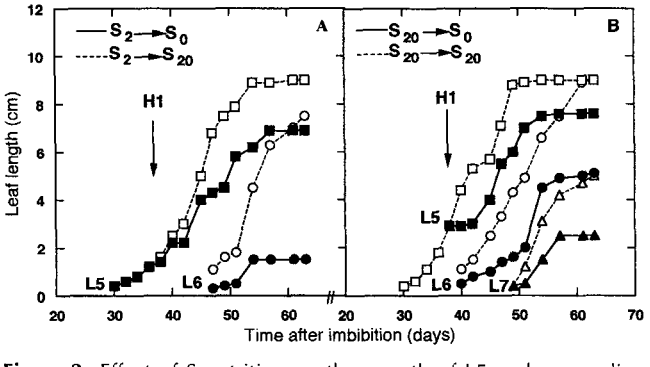

Figure 2. Effect of S nutrition on the growth of L5 and succeeding leaves during the course of the experiment. A, Plants grown initially at S_2 and then transferred to solution without sulfate ($S_2 \rightarrow S_0$, closed symbols) or S_{20} ($S_2 \rightarrow S_{20}$, open symbols). B, Plants grown initially at S_{20} and then transferred to solution without sulfate ($S_{20} \rightarrow S_0$, closed symbols) or maintained in S_{20} ($S_{20} \rightarrow S_{20}$, open symbols).

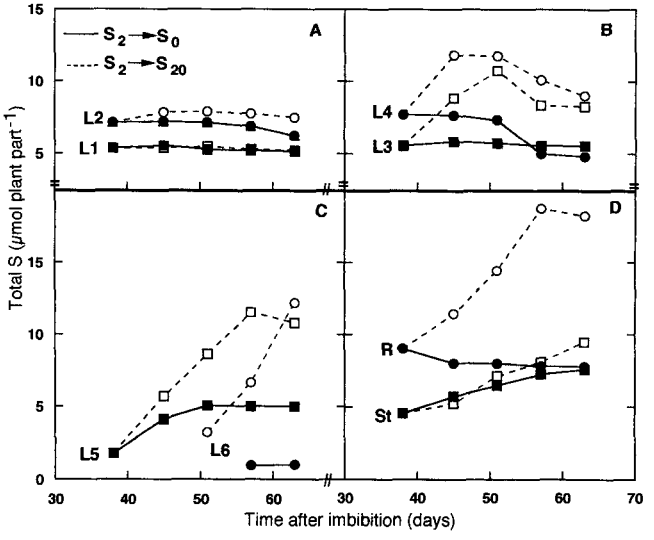

Figure 3. S content of the individual parts of plants grown initially at S_2 and then transferred to medium without sulfate ($S_2 \rightarrow S_0$, closed symbols) or with S_{20} ($S_2 \rightarrow S_{20}$, open symbols). A, L1 and L2. B, L3 and L6. C, L5 and L6. D, Root (R) and stem (St).

The distribution of ^{35}S label in S_{20} plants at H1 was altogether different from that in S_2 plants. Most of the label was associated with the expanding L4 (Table IV), mostly in the soluble fraction (Table V), and in the root system (Table IV), where about 60% of it was associated with the insoluble fraction (Table V). Smaller amounts of label occurred in most of the other plant parts, variously distributed between the soluble and insoluble fractions.

Effect of S Nutrition on the S Content of Plants Grown Initially at S_2

The S content of L1 to L3 of $S_2 \rightarrow S_0$ plants, which were fully expanded when the supply of sulfate was terminated at d 38, remained relatively constant for the remainder of the experiment (Fig. 3). However, after the S supply was discontinued, the S content of L4 and the root system decreased by 38 and 15%, respectively. Presumably this S was redistributed to other plant parts via the recycling pool of S described by Larsson et al. (1991). The losses from L4 and the root were offset by net gains in L5 and the stem and, to a very small extent, L6. The data in Table II show that the S gained by L5 and the stem was accompanied by a quantitatively similar loss of soluble S in L4 and the root and that losses of S from the insoluble fraction of the constituent parts of $S_2 \rightarrow S_0$ plants throughout the experiment were negligible. Conversely, the S imported into L5 and the stem was incorporated into the insoluble fraction.

Table II. *Partitioning of S and ^{35}S label between the soluble and insoluble fractions of L3 to L5, the stem, and the roots of S_2, $S_2 \rightarrow S_0$, and $S_2 \rightarrow S_{20}$ plants*

The values given at H1 are for S_2 plants immediately prior to initiating the $S_2 \rightarrow S_0$ and $S_2 \rightarrow S_{20}$ treatments (see Fig. 1).

Plant Part	Harvest	S Content (mean ± SE)				^{35}S Label (mean ± SE)			
		$S_2/S_2 \rightarrow S_0$		$S_2/S_2 \rightarrow S_{20}$		$S_2/S_2 \rightarrow S_0$		$S_2/S_2 \rightarrow S_{20}$	
		Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
		μmol (plant part)$^{-1}$				*kBq (plant part)$^{-1}$*			
L3	H1	0.0 ± 0.0	5.5 ± 0.1	0.0 ± 0.0	5.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
	H3	0.2 ± 0.0	5.5 ± 0.1	3.4 ± 0.1	7.4 ± 0.3	0.0 ± 0.0	0.2 ± 0.0	2.4 ± 0.1	4.0 ± 0.2
	H5	0.0 ± 0.0	5.5 ± 0.2	0.2 ± 0.0	8.1 ± 0.4	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	4.0 ± 0.1
L4	H1	2.8 ± 0.1	4.9 ± 0.2	2.8 ± 0.1	4.9 ± 0.2	1.3 ± 0.0	0.6 ± 0.0	1.3 ± 0.0	0.6 ± 0.0
	H3	1.0 ± 0.0	6.3 ± 0.3	5.7 ± 0.3	6.1 ± 0.3	0.5 ± 0.0	0.6 ± 0.0	5.9 ± 0.2	7.1 ± 0.3
	H5	0.0 ± 0.0	4.8 ± 0.3	0.8 ± 0.0	8.2 ± 0.3	0.0 ± 0.0	0.6 ± 0.0	0.5 ± 0.2	7.5 ± 0.3
L5	H1	0.8 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	H3	0.1 ± 0.0	5.0 ± 0.2	3.6 ± 0.1	5.0 ± 0.2	0.7 ± 0.0	9.2 ± 0.4	14.0 ± 0.5	20.5 ± 1.1
	H5	0.0 ± 0.0	5.0 ± 0.2	2.7 ± 0.1	8.1 ± 0.3	0.5 ± 0.0	8.8 ± 0.4	5.4 ± 0.2	15.1 ± 0.7
Stem	H1	1.4 ± 0.0	3.2 ± 0.1	1.4 ± 0.0	3.2 ± 0.1	1.0 ± 0.0	0.3 ± 0.0	1.0 ± 0.0	0.3 ± 0.0
	H3	1.5 ± 0.0	5.0 ± 0.2	2.5 ± 0.1	4.6 ± 0.2	2.0 ± 0.1	4.9 ± 0.2	2.1 ± 0.1	3.9 ± 0.2
	H5	1.5 ± 0.0	6.1 ± 0.3	2.1 ± 0.1	7.4 ± 0.3	0.8 ± 0.0	6.9 ± 0.3	2.5 ± 0.1	4.5 ± 0.2
Root	H1	1.5 ± 0.0	7.5 ± 0.3	1.5 ± 0.0	7.5 ± 0.3	63.9 ± 3.2	30.3 ± 1.5	63.9 ± 3.2	30.3 ± 1.5
	H3	0.4 ± 0.0	7.5 ± 0.4	6.9 ± 0.3	7.5 ± 0.3	30.2 ± 1.6	50.3 ± 2.4	28.1 ± 1.3	30.3 ± 1.4
	H5	0.1 ± 0.0	7.7 ± 0.3	4.6 ± 0.2	13.7 ± 0.5	0.3 ± 0.0	78.0 ± 4.5	1.1 ± 0.0	33.2 ± 1.7

With the exception of L1, the S content of all of the plant parts increased, at least in the short term, when S_2 plants were transferred to S_{20} (Fig. 3). These increases occurred even in the fully expanded L2 and, more particularly, in L3. The short-term increases in the S content of L2 to L4 in $S_2 \rightarrow S_{20}$ plants were in marked contrast to the steady-state levels in these leaves in $S_2 \rightarrow S_0$ plants. Similarly, the root, which underwent net loss of S in $S_2 \rightarrow S_0$ plants, exhibited a large net gain. In the expanding L5 and L6, the increase in the S content of $S_2 \rightarrow S_{20}$ plants was greater than in plants without sulfate, largely due to increased growth, since the S content per gram dry weight was proportionately not as much in the plants transferred to S_{20} (27.6 μmol g^{-1}) as those without sulfate (20.3 μmol g^{-1}). In the longer term (after H3), the S content of L2 to L4 of plants transferred to S_{20} declined markedly (Fig. 3). This was also incipient in L5 at the end of the experiment. These net losses are consistent with the characteristics of mature S-sufficient leaves (Adiputra and Anderson, 1992, 1995; Sunarpi and Anderson, 1996).

In plants that were transferred to S_{20}, the level of both soluble and insoluble S at H3 was enhanced in all of the plant parts examined (Table II). This included the fully expanded L3, in which 35% of the increase in the S content between H1 and H3 occurred in the insoluble fraction. The analogous value for L4, which was 70% expanded at H1, was 30%. The data in Table II also show that the longer term net losses of S from L3 and L4 after H3 in $S_2 \rightarrow S_{20}$

Table III. *Sulfate S in the soluble fraction of L3 to L5, the stem, and the root system in S_2 and S_{20} plants at harvest H1, and $S_2 \rightarrow S_0$, $S_2 \rightarrow S_{20}$, $S_{20} \rightarrow S_0$, and $S_{20} \rightarrow S_{20}$ plants at H3 and H5*

The values for soluble S are given for the S_2 series in Table II and for the S_{20} series in Table V.

Plant Part	Harvest	Sulfate Content (mean ± SE)			
		$S_2/S_2 \rightarrow S_0$	$S_2/S_2 \rightarrow S_{20}$	$S_{20}/S_{20} \rightarrow S_0$	$S_{20}/S_{20} \rightarrow S_{20}$
		μmol (plant part)$^{-1}$			
L3	H1	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
	H3	0.1 ± 0.0	2.6 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
	H5	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
L4	H1	2.3 ± 0.1	2.3 ± 0.1	7.3 ± 0.3	7.3 ± 0.3
	H3	0.7 ± 0.0	4.3 ± 0.2	1.7 ± 0.1	2.9 ± 0.1
	H5	0.0 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
L5	H1	0.7 ± 0.0	0.7 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
	H3	0.1 ± 0.0	2.7 ± 0.1	0.6 ± 0.0	1.5 ± 0.1
	H5	0.0 ± 0.0	2.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
Stem	H1	1.2 ± 0.0	1.2 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
	H3	1.1 ± 0.1	1.9 ± 0.1	1.1 ± 0.0	1.8 ± 0.1
	H5	1.3 ± 0.1	1.9 ± 0.1	1.3 ± 0.0	1.3 ± 0.1
Root	H1	1.3 ± 0.0	1.3 ± 0.0	4.6 ± 0.2	4.6 ± 0.2
	H3	0.3 ± 0.0	5.1 ± 0.2	1.9 ± 0.1	4.9 ± 0.2
	H5	0.1 ± 0.0	3.6 ± 0.2	1.2 ± 0.1	4.0 ± 0.2

Table IV. *Time course of the ^{35}S-label associated with the individual parts of plants grown initially at S_2 or S_{20} and then transferred to medium without sulfate ($S_2 \rightarrow S_0$, $S_{20} \rightarrow S_0$) or with S_{20} ($S_2 \rightarrow S_{20}$, $S_{20} \rightarrow S_{20}$)*

[^{35}S]Sulfate (2 μM, specific radioactivity 185 GBq mol^{-1}, or 20 μM, specific radioactivity 18.5 GBq mol^{-1}) was supplied immediately prior to H1 as detailed in Figure 1. The values at H1 apply to S_2 and S_{20} plants immediately prior to initiating the $S_2 \rightarrow S_0$, $S_2 \rightarrow S_{20}$, $S_{20} \rightarrow S_0$, and $S_{20} \rightarrow S_{20}$ treatments. For the plants grown initially at S_2, the label in L1 and L2 did not exceed 0.2 kBq leaf^{-1} for any of the treatments at any harvest and the values have been omitted. The analogous value for plants grown initially at S_{20} was 0.9 kBq leaf^{-1}.

Plant Part	Harvest	^{35}S Label (mean ± SE)			
		Plants initially at S_2		Plants initially at S_{20}	
		$S_2/S_2 \rightarrow S_0$	$S_2/S_2 \rightarrow S_{20}$	$S_{20}/S_{20} \rightarrow S_0$	$S_{20}/S_{20} \rightarrow S_{20}$
		kBq (plant part)$^{-1}$			
L3	H1	0.4 ± 0.0	0.4 ± 0.0	8.1 ± 0.3	8.1 ± 0.3
	H2	0.2 ± 0.0	5.3 ± 0.2	6.6 ± 0.3	5.7 ± 0.2
	H3	0.2 ± 0.0	6.4 ± 0.3	6.4 ± 0.3	3.5 ± 0.1
	H4	0.2 ± 0.0	4.2 ± 0.2	6.3 ± 0.2	3.5 ± 1.6
	H5	0.2 ± 0.0	4.0 ± 0.1	6.0 ± 0.2	3.5 ± 0.2
L4	H1	1.9 ± 0.1	1.9 ± 0.1	51.3 ± 2.3	51.3 ± 2.3
	H2	1.1 ± 0.0	12.6 ± 0.5	33.9 ± 1.6	30.4 ± 1.6
	H3	1.1 ± 0.0	13.1 ± 0.4	14.3 ± 0.6	3.1 ± 0.6
	H4	0.7 ± 0.0	11.0 ± 0.5	13.4 ± 0.5	8.2 ± 0.4
	H5	0.6 ± 0.0	8.0 ± 0.4	12.9 ± 0.5	7.0 ± 0.3
L5	H1	0.2 ± 0.0	0.2 ± 0.0	5.7 ± 0.2	5.7 ± 0.2
	H2	9.5 ± 0.4	19.6 ± 0.9	24.6 ± 1.2	29.4 ± 1.4
	H3	9.9 ± 0.5	34.6 ± 1.5	43.7 ± 2.1	44.4 ± 2.3
	H4	9.7 ± 0.5	42.5 ± 2.5	32.3 ± 1.4	12.3 ± 0.6
	H5	9.2 ± 0.5	20.5 ± 1.2	27.9 ± 1.3	10.0 ± 0.4
L6	H1	ndv[a]	ndv	ndv	ndv
	H2	ndv	ndv	ndv	0.8 ± 0.0
	H3	ndv	5.8 ± 0.2	0.9 ± 0.0	6.4 ± 0.3
	H4	0.4 ± 0.0	16.5 ± 0.8	3.5 ± 0.2	34.2 ± 1.6
	H5	0.4 ± 0.0	36.1 ± 1.7	9.4 ± 0.3	25.1 ± 1.2
Stem	H1	1.3 ± 0.0	1.3 ± 0.0	7.3 ± 0.3	7.3 ± 0.3
	H2	4.9 ± 0.2	5.7 ± 0.2	13.2 ± 0.6	14.0 ± 0.5
	H3	6.9 ± 0.3	6.0 ± 0.3	10.7 ± 0.4	14.4 ± 0.6
	H4	7.5 ± 0.3	7.0 ± 0.4	13.6 ± 0.6	14.5 ± 0.7
	H5	7.7 ± 0.3	7.0 ± 0.4	14.5 ± 0.7	14.0 ± 0.7
Root	H1	94.2 ± 3.6	94.2 ± 3.6	41.1 ± 2.4	41.1 ± 2.4
	H2	81.2 ± 4.1	68.9 ± 3.4	33.6 ± 1.4	30.5 ± 1.4
	H3	80.5 ± 4.3	58.4 ± 2.5	32.4 ± 1.4	26.5 ± 1.3
	H4	78.4 ± 4.0	35.0 ± 1.7	32.7 ± 1.6	24.5 ± 1.6
	H5	78.3 ± 3.6	34.3 ± 0.7	31.2 ± 1.5	24.5 ± 1.2

[a] ndv, Not developed.

plants (Fig. 3) must occur from the soluble fraction, since the insoluble fraction in these leaves continued to increase during the period of net S loss. Indeed, for L3 the level of soluble S decreased to a very low level by the end of experiment (H5). Since sulfate was the principal constituent of the soluble fraction at H3 (see legend to Table III) when the soluble S pool in L3 and L4 was maximal (Table II), this suggests that sulfate was the principal direct or indirect source of the S exported from these leaves in the period following H3.

Effect of S Nutrition on the Distribution of ^{35}S Label in Plants Grown Initially at S_2

The ^{35}S label that was distributed to various parts of the plants during the pulse with 2 μM [^{35}S]sulfate (see values at H1, Table IV) was subsequently redistributed to other plant parts. The nature of the redistribution was highly influenced by the level of S nutrition during the chase period. In the absence of exogenous sulfate ($S_2 \rightarrow S_0$), the redistribution of ^{35}S label was limited to a small net loss from the root system and extremely small losses from L2, L3, and L4. The cumulative losses from these sources were offset by increases in the developing L5 and to a lesser extent in the stem. The data in Table II indicate that the large pool of ^{35}S label in the soluble fraction of the root at the end of the chase period quantitatively accounted for most of the increases observed in other fractions and other plant parts. A substantial proportion (75%) of the label lost from the soluble fraction of the root was incorporated into the insoluble fraction of the root over the course of the experiment; the balance was recovered in the insoluble fraction in the stem and L5.

The redistribution of ^{35}S label was both qualitatively and quantitatively very different in plants that were transferred to S_{20} ($S_2 \rightarrow S_{20}$). L3-L4, which exported ^{35}S when S_2 plants

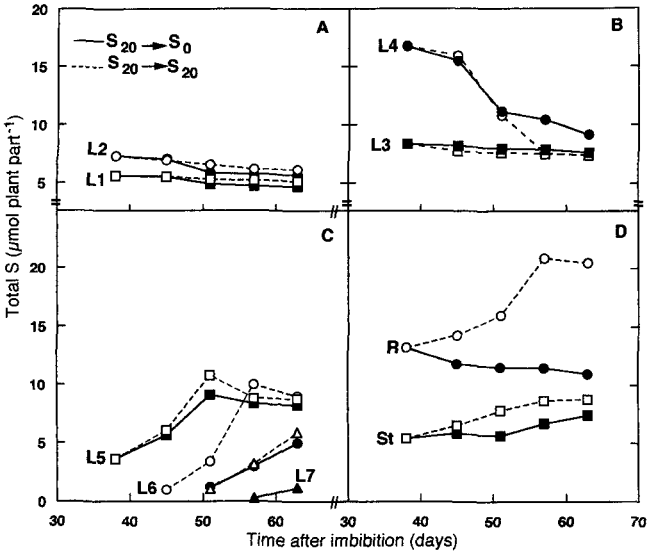

Figure 4. S content of the individual parts of plants grown initially at S_{20} and then transferred to medium without sulfate ($S_{20} \rightarrow S_0$, closed symbols) or maintained in S_{20} ($S_{20} \rightarrow S_{20}$, open symbols). A, L1 and L2. B, L3 and L4. C, L5, L6 and L7. D, Root (R) and stem (St).

were transferred to sulfate-free medium, exhibited massive short-term import of ^{35}S during the chase period when transferred to S_{20} (Table IV). This effect was also evident, albeit at a low level, in L2. Transfer to S_{20} also promoted import of label into the developing L5 and L6. Conversely, these conditions promoted massive export (95%) of label from the soluble fraction of the root system, which is in sharp contrast to the large incorporation of ^{35}S label from the soluble fraction of the root into the insoluble fraction of the root in $S_{20} \rightarrow S_0$ plants. At H3 a significant proportion (37–45%) of the label in $S_2 \rightarrow S_{20}$ plants was associated with the soluble fraction of L3 to L5 (Table II). However, very little label was incorporated into the insoluble fraction of L3 and L4, which had attained full expansion at H3. In the expanding L5, this was associated with an increase in the label in the insoluble fraction at H5. Following H3 the amount of label in the soluble fraction of these leaves declined; presumably the balance of the label was redistributed to other plant parts, which were not analyzed for soluble and insoluble S (Table II) such as L6, the stem, and the root, as indicated by the data in Table IV.

Effect of S Nutrition on the S Content of Plants Grown Initially at S_{20}

Plants that were maintained at S_{20} throughout the experiment ($S_{20} \rightarrow S_{20}$) exhibited long-term loss of S from the fully expanded L1 to L3 at a very slow rate (Fig. 4). The net loss of S from L4 in the period following H1 (when it was 70% expanded) is consistent with the S status of a S-sufficient soybean leaf as it approaches and attains full expansion, as is the sequential import (and subsequent export) of S into L5 to L8 (Fig. 4) as each of these leaves develops and matures (Sunarpi and Anderson, 1996). The gradual increase in the S content in the root and the stem is also consistent with S-sufficient growth. The data in Table V indicate that the amount of S in the soluble fraction of leaves of $S_{20} \rightarrow S_{20}$ plants decreases as they mature. Since the insoluble fraction in these leaves shows little if any decrease during this period, the net loss of total S must involve export of S from the soluble fraction. The maintenance of a substantial pool of soluble S in the root of $S_{20} \rightarrow S_{20}$ plants throughout the experiment is consistent with the role of this organ in the acquisition of S from the nutrient solution and incorporation of a proportion of the acquired S into the insoluble fraction of a growing root system. The data in Table III indicate that sulfate was the principal form of S in the root.

The S content of L1 to L5 was not greatly affected by discontinuing the S supply ($S_{20} \rightarrow S_0$), although there was an indication of enhanced loss of S from L4 and diminished import into L5 compared with plants maintained on S_{20} ($S_{20} \rightarrow S_{20}$). However, the S content of the leaves that subsequently formed was highly sensitive to the curtailment of the S supply (Fig. 4). Terminating the S supply ($S_{20} \rightarrow S_0$) caused prompt cessation in the gain of S by the roots; indeed, the root system underwent continued net loss of S, albeit at a slow rate, during the experiment. Since the plants had no access to exogenous S during the experiment, the data imply that L4 was the principal source of the S imported into L5 and, subsequently, L6. Clearly, the S that was redistributed from L4 was more or less able to sustain the S requirement associated with the growth of L5, but it was clearly insufficient to support optimum growth of L6. The fractionation data (Table V) indicate that partitioning of S between the soluble and the insoluble fractions of L3 to L5 was not greatly affected by terminating the external S supply; any differences between $S_2 \rightarrow S_0$ and $S_2 \rightarrow S_{20}$ plants were restricted to somewhat lower levels of S in the soluble fraction in L4 and L5 at H3 of $S_2 \rightarrow S_{20}$ plants. For the plants grown without added S, the data imply that the large gain in insoluble S in L5 between H1 and H3 was due to redistribution of S from the soluble fraction of L4. When data from plants grown with and without S_{20} were compared, there was no evidence that S deprivation enhanced the loss of S from the insoluble fraction of L3 to L5, although the levels of S in the soluble fraction of these leaves were somewhat lower than those in S-sufficient plants. On the other hand, S deprivation almost totally inhibited the incorporation of S into the insoluble pool of the root and promoted the depletion of the soluble pool, most of which was exported to other plant parts.

Effect of S Nutrition on the Distribution of ^{35}S Label in Plants Grown Initially at S_{20}

The labeling patterns of plants previously grown at S_{20} were markedly different from those grown at S_2 (Table IV). L4, which was the dominant short-term sink for S supplied to S_{20} plants during the pulse, underwent massive loss of label during the chase period. This loss was not influenced by the level of S nutrition, except in the later stages of the experiment when there was some evidence that ^{35}S was preferentially retained in plants without exogenous S (Table IV). The ^{35}S label exported from L4 was imported into L5. However, whereas most of the label that was imported

Table V. *Partitioning of S and ^{35}S label between the soluble and insoluble fractions of L3 to L5, the stem, and the root system of S_{20}, $S_{20}\rightarrow S_0$ and $S_{20}\rightarrow S_{20}$ plants*

The values at H1 are for S_{20} plants immediately prior to initiating the $S_{20}\rightarrow S_0$ and $S_{20}\rightarrow S_{20}$ treatments (see Fig. 1).

Plant Part	Harvest	S Content (mean ± SE)				^{35}S Label (mean ± SE)			
		$S_{20}/S_{20}\rightarrow S_0$		$S_{20}\rightarrow S_{20}$		$S_{20}/S_{20}\rightarrow S_0$		$S_{20}\rightarrow S_{20}$	
		Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
		μmol (plant part)$^{-1}$				*kBq (plant part)$^{-1}$*			
L3	H1	1.1 ± 0.0	7.2 ± 0.4	1.1 ± 0.0	7.2 ± 0.4	4.6 ± 0.2	3.5 ± 0.1	4.6 ± 0.2	3.5 ± 0.1
	H3	0.2 ± 0.0	7.4 ± 0.3	0.3 ± 0.0	7.6 ± 0.3	0.4 ± 0.0	6.0 ± 0.3	0.1 ± 0.0	3.5 ± 0.1
	H5	0.3 ± 0.0	7.1 ± 0.3	0.3 ± 0.0	7.4 ± 0.3	0.1 ± 0.0	5.9 ± 0.2	0.1 ± 0.0	3.4 ± 0.1
L4	H1	9.1 ± 0.4	7.7 ± 0.4	9.1 ± 0.4	7.7 ± 0.4	44.3 ± 2.3	7.0 ± 0.3	44.3 ± 2.3	7.0 ± 0.3
	H3	2.4 ± 0.1	8.4 ± 0.4	3.0 ± 0.1	8.1 ± 0.4	1.4 ± 0.0	12.9 ± 0.7	6.1 ± 0.3	7.0 ± 0.3
	H5	0.2 ± 0.0	7.2 ± 0.3	0.3 ± 0.0	8.9 ± 0.4	0.2 ± 0.0	12.7 ± 0.5	0.4 ± 0.0	6.6 ± 0.3
L5	H1	1.6 ± 0.0	2.0 ± 0.1	1.6 ± 0.0	2.0 ± 0.1	2.5 ± 0.1	3.2 ± 0.1	2.5 ± 0.1	3.2 ± 0.1
	H3	0.9 ± 0.0	8.2 ± 0.4	2.0 ± 0.0	8.8 ± 0.4	15.9 ± 0.9	27.9 ± 1.1	34.4 ± 1.5	10.0 ± 0.5
	H5	0.6 ± 0.0	7.6 ± 0.3	0.7 ± 0.0	8.1 ± 0.4	0.7 ± 0.0	27.2 ± 1.3	0.8 ± 0.0	9.3 ± 0.5
Stem	H1	2.0 ± 0.1	3.4 ± 0.1	2.0 ± 0.1	3.4 ± 0.1	4.2 ± 0.2	3.0 ± 0.1	4.2 ± 0.2	3.0 ± 0.1
	H3	1.5 ± 0.1	4.1 ± 0.2	2.4 ± 0.1	5.4 ± 0.2	2.5 ± 0.1	8.2 ± 0.4	2.4 ± 0.1	12.1 ± 0.5
	H5	1.6 ± 0.1	5.8 ± 0.2	1.6 ± 0.1	7.2 ± 0.3	2.3 ± 0.1	12.1 ± 0.5	2.7 ± 0.1	11.3 ± 0.5
Root	H1	5.7 ± 0.2	7.5 ± 0.3	5.7 ± 0.2	7.5 ± 0.3	16.6 ± 0.9	24.4 ± 1.1	16.6 ± 0.9	24.4 ± 1.1
	H3	2.7 ± 0.1	8.8 ± 0.4	7.0 ± 0.3	9.0 ± 0.4	4.1 ± 0.2	28.2 ± 1.4	2.1 ± 0.1	24.4 ± 1.2
	H5	1.4 ± 0.0	9.5 ± 0.4	5.1 ± 0.2	15.4 ± 0.8	0.2 ± 0.0	31.0 ± 1.5	0.2 ± 0.0	24.2 ± 0.1

into L5 in S-sufficient plants ($S_{20} \rightarrow S_{20}$) was promptly re-exported a second time into L6, the loss of label from plants transferred to S-deficient medium ($S_{20} \rightarrow S_0$) was greatly diminished, as was the gain of label by L6 in these plants. Similarly, a higher proportion of label was retained in the root under S-insufficient conditions.

The data in Table V indicate that most of the label (about 86%) delivered to L4 during the pulse period occurred in the soluble fraction and this was the principal source of the label imported into other plant parts during the chase. In the $S_{20} \rightarrow S_0$ plants a small amount of label was incorporated into the insoluble fraction but this did not occur in S-sufficient plants. In both plants most of the label from the soluble fraction of L4 was delivered to L5 in the period between H1 and H3. In the $S_{20} \rightarrow S_0$ plants most of the label imported into L5 (63%) was incorporated into the insoluble fraction and remained associated with this fraction for the remainder of the experiment. In the $S_{20} \rightarrow S_{20}$ plants, however, only about 22% of the label was incorporated into the insoluble fraction of L5. Importantly, the label that was not incorporated into the soluble fraction of L5 by H3 was exported, almost in its entirety, during the period between H3 and H5, presumably to L6 (Table IV). Essentially similar remarks apply to the small amount of ^{35}S label that was delivered to L3 during the pulse, albeit at a much lower level. Most of the ^{35}S label in the soluble fraction of L3 of $S_{20} \rightarrow S_0$ plants at H1 was incorporated into the insoluble fraction and remained there for the rest of the experiment; the balance of the S in the soluble fraction of L3 was exported. However, in $S_{20} \rightarrow S_{20}$ plants the label in the soluble fraction at H1 was exported in its entirety, which suggests that L3, even when fully expanded, had a small requirement to incorporate S into the insoluble fraction, which in S-insufficient plants could only be supplied from within the leaf. The decrease in the ^{35}S label from the root of $S_{20} \rightarrow S_0$ and $S_{20} \rightarrow S_{20}$ plants was due to the loss of ^{35}S label from the soluble fraction; the slightly smaller net decrease from the root of S-deprived plants was attributed to the incorporation of a small amount of label into the insoluble fraction in the root.

Specific Radioactivity of the Plant Parts during the Chase Period

In $S_2 \rightarrow S_0$ plants the specific radioactivity of the roots remained essentially constant (Fig. 5), indicating that neither S nor ^{35}S were obtained from other plant parts. On the other hand, the massive decreases in the specific radioactivity of the roots of $S_2 \rightarrow S_{20}$ plants represent both net gain of S from the nutrient solution and export of ^{35}S label to the leaves and the stem. Conversely, the very low specific radioactivity of the leaves of $S_2 \rightarrow S_0$ plants results from the

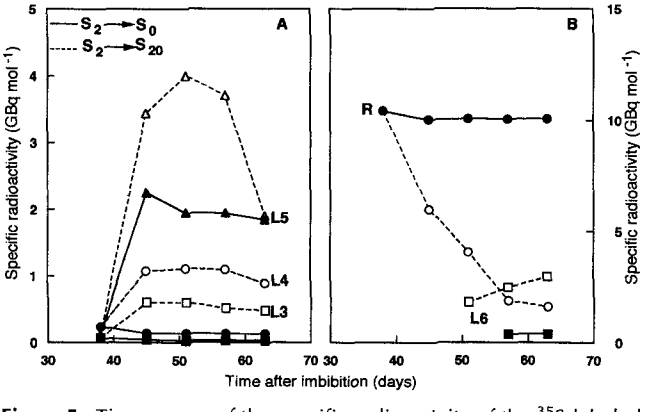

Figure 5. Time course of the specific radioactivity of the ^{35}S-labeled S associated with L3 to L6 and the root system during the chase period in $S_2 \rightarrow S_0$ plants (closed symbols) and $S_2 \rightarrow S_{20}$ plants (open symbols). Note the different scales for specific radioactivity for L3 to L5 (A) and the root and L6 (B). R, Root.

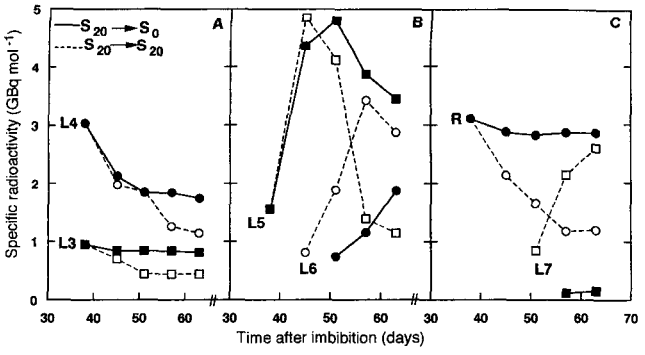

Figure 6. Time course of the specific radioactivity of the ^{35}S-labeled S associated with L3 to L7 and the root system during the chase period in $S_{20} \rightarrow S_0$ plants (closed symbols) and $S_{20} \rightarrow S_{20}$ plants (open symbols). R, Root.

retention of ^{35}S label in the roots. The very large increase in the specific radioactivity of L3 to L5 of $S_2 \rightarrow S_{20}$ plants, even though they acquired a large amount of S from the nutrient solution, reflects the gain of ^{35}S label from the soluble fraction of the roots.

The changes in the specific radioactivity of the leaves of the $S_{20} \rightarrow S_{20}$ plants (Fig. 6) result from the import of ^{35}S from the soluble fraction of the roots and its passage, initially into L4, then L5, and eventually L6. The concomitant decrease in the specific radioactivity of L5 in the period after H2, with the increase in the specific radioactivity of L6, was a consequence of several factors. First, relatively little label was incorporated into the insoluble fraction of L5 during this period. Second, the label that was delivered to L5 during the chase was mostly restricted to the soluble fraction and was selectively redistributed to L6 without equilibrating with the insoluble S pool of L5, hence, the gradual increase in the specific radioactivity of L6. Third, it appears that the labeled S that entered the soluble fraction of L5 during the early stages of the experiment (up to d 45) was more or less segregated from the unlabeled S that continued to be delivered to L5 in the subsequent period. This could involve compartmentation of S within the soluble fraction (Anderson, 1990). For example, labeled S could be delivered to a small compartment (e.g. cytoplasm) that is not in rapid equilibrium with the bulk of the unlabeled S in the soluble fraction (e.g. vacuolar sulfate). The labeled S is then exported without mixing with the large compartment. Prompt export from a small pool would minimize dilution of ^{35}S by incoming unlabeled S. The smaller decreases in the specific radioactivity in the leaves of the $S_{20} \rightarrow S_0$ plants presumably reflect gradual cessation of delivery of unlabeled S from the nutrient solution, thereby leading to cessation of redistribution from the soluble fraction of these leaves.

DISCUSSION

The data for the S content of the leaves of soybean plants grown under S-sufficient conditions ($S_{20} \rightarrow S_{20}$) confirm and extend the results reported previously (Sunarpi and Anderson, 1996). After leaves attain about 70% of full expansion, the S content of the insoluble fraction undergoes very little if any net loss of S, whereas the S content of the soluble fraction declines over an extended period, almost to zero. The possibility of rapid turnover of S between the insoluble and soluble fraction in mature leaves during the period of net S loss is ruled out by the labeling data, which indicate that any ^{35}S label incorporated into the insoluble fraction remains associated with the insoluble fraction during the life of the leaf, regardless of how early or late in the development the ^{35}S label is incorporated. Conversely, almost all of the ^{35}S label that is not incorporated into the insoluble fraction is exported, largely as sulfate. This is consistent with current knowledge of sulfate efflux from the vacuoles of mesophyll cells (Bell et al., 1990). The data therefore indicate that the export of S from the leaves of S-sufficient plants is most active after the leaf achieves 70% of full expansion (Sunarpi and Anderson, 1996). This presumably coincides with a large decrease in the demand of the leaf for S for growth. However, it is not clear whether the low level of export prior to this time results from a relatively stronger demand for S for leaf growth and/or low level activity of the exporting mechanism.

Termination of the exogenous S source causes only minor modification to the processes as detailed above for S-sufficient plants. The data in Tables II and V indicate that the imposition of S stress (i.e. $S_2 \rightarrow S_0$, $S_{20} \rightarrow S_0$) promotes the remobilization of a small amount of S (<2%) from the insoluble fraction of mature leaves. However, this is not evident in the corresponding ^{35}S-labeling data (Tables II and V), perhaps implying that the S that is incorporated into the insoluble fraction (protein and sulfolipid) during the early stages of leaf development is degraded preferentially. With this exception, the ability of a plant to sustain growth in the absence of an external source of S is largely determined by the size of the endogenous soluble S pool in the root and in leaves that are greater than 50% expanded (or have recently attained full expansion), since these structures are the principal sources of S that are directed into new growth.

In $S_{20} \rightarrow S_0$ plants the large amount of ^{35}S label incorporated into the insoluble fraction of developing plant parts (e.g. L4 and L5), compared with plants maintained under S-sufficient conditions ($S_{20} \rightarrow S_{20}$), is attributed to the dynamics of the labeled soluble pool of S available for incorporation into the insoluble fraction. In $S_{20} \rightarrow S_0$ plants the labeled pool in the leaves represents the only source of S available, whereas in $S_{20} \rightarrow S_{20}$ plants the labeled S in the soluble fraction of leaves competes with unlabeled S acquired from the nutrient solution.

Growing structures have a constitutive demand for S to synthesize protein and sulfolipid during growth. A small amount of S is also required in mature structures for the turnover of various, essential S-containing molecules. In theory, S accumulates in the soluble fraction of a growing structure if the delivery of S from the other plant parts and from the nutrient solution exceeds the incorporation into the insoluble fraction and the re-export of S. However, none of these processes appear to be responsive to signals from the internal S pools. Rather, the fluctuation in the size of the soluble pool seems to be determined mainly by the availability of exogenous S for distribution (and subsequent redistribution) and

by competition between processes for soluble S. The latter is shown by the net long-term incorporation of S into the insoluble fraction of mature L3 and L4 when S-limited plants were transferred to S-sufficient conditions (i.e. $S_2 \rightarrow S_{20}$, Table II), whereas plants that were transferred to medium without S ($S_2 \rightarrow S_0$) exhibited growth of L5 at the expense of the soluble fraction of L4. This implies that, in an incompletely expanded leaf of a plant grown under S-limiting conditions, the demand for S within the leaf for the synthesis of essential molecules for growth (e.g. protein) competes with the mechanism for the re-export of S to other less-well developed structures. It appears that, if the S demand of a leaf is not fulfilled during growth because of the competing export process, the unfulfilled requirement for S for incorporation into the insoluble fraction can, within limits, be satisfied at some later time if the S supply is reinstigated, i.e. S insufficiency in a leaf can be reversed in the short to medium term, as revealed by the S status of L3 and L4 in $S_2 \rightarrow S_{20}$ plants.

The relative insensitivity of the insoluble pool of mature leaves to the imposition of S stress is consistent with that found in earlier studies of total leaf S in subterranean clover (Bouma, 1966) and barley (Adiputra and Anderson, 1995). Collectively, these studies are at variance with that by Bell et al. (1995), who reported that the insoluble S fraction of mature leaves of siratro decreased by about 50% 3 d after the supply of sulfate was discontinued. We wonder whether the N:S ratio of 20, used in the nutrient solution by Bell et al. (1995), and which approximates the N:S ratio found in plants, promotes remobilization of insoluble N (i.e. protein) and, indirectly, remobilization of S. On the other hand, the N:S ratios (750–7500) used in the experiment in this study might be less conducive to inducing protein remobilization.

The S status of the root system was very dependent on the level of S nutrition. The data for the $S_2 \rightarrow S_0$ plants indicate that, whereas incorporation of S into the insoluble fraction of the developing leaf L5 was maintained when the S supply was discontinued (Table II), S incorporation into the insoluble fraction of the root system ceased abruptly. At the same time, the labeling data for the $S_2 \rightarrow S_0$ plants indicate that most of the S in the soluble fraction of the roots was retained in the root and incorporated into the insoluble fraction. Conversely, labeled S was readily chased out of the soluble fraction of the roots upon the addition of exogenous S as evidenced by the large loss of label from the roots and the short-term increase in the label in L3 to L6 in $S_2 \rightarrow S_{20}$ plants. The export of ^{35}S label in this way indicates that the ^{35}S label in the soluble fraction of the root did not undergo extensive dilution with the newly acquired S from the nutrient solution. This provides further evidence in support of two compartments within the soluble fraction, one which appears to be relatively small and metabolically active and the other that is large and in slow equilibrium with the small compartment, presumably corresponding with the cytoplasm and vacuole, respectively.

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